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13. ABSTRACT *(Maximum 200 Words)*

This concept award was extended by a year over the initial one-year period at no additional cost. The primary objective is to investigate liposomal encapsulation of chelated Ac-225 in order to reduce loss of progeny from the targeting vehicle and, therefore, the tumor site. Results have shown that the extent of daughter retention is dependent upon liposomal vesicle diameter with larger vesicles retaining the alpha-particle emitting daughters to a greater extent. The dependence of retention on the diameter of the liposomal vesicle is thought to arise because of recoil of the daughter nuclei. The recoil distance of daughter nuclei after alpha-particle emission is approximately 100 nm. Depending upon the site of decay, therefore, the recoil distance is sufficient to eject the daughter outside of the liposome. Assuming that ejection by recoil is the only mechanism by which daughters escape from the vesicles, a theoretical model relating vesicular diameter with daughter retention has been developed. Measured retention values do not fall within model predictions. Upcoming studies will focus on understanding the observed divergence between model-predicted and observed retention and also on carrying out animal studies evaluating loco-regional therapy with larger vesicles.

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Table of Contents

INTRODUCTION

Alpha particle-emitting radionuclides hold great promise as potential therapeutic agents for cancer treatment (1). To date, only 2 such radionuclides, astatine-211 (At-211) and bismuth-213 (Bi-213) have been investigated clinically. Both have short half-lives (7 h and 46 min, respectively) and are, therefore, appropriate primarily for situations in which targeting is very rapid. Their use has been limited to leukemia patients, in which the disease is rapidly accessible by intravenous administration (Bi-213) (2) or to patients that have undergone surgical excision of brain cancer and the radionuclide is injected into the surgical cavity (At-211). The former study demonstrated low toxicity of IV-injected alpha emitters, the latter approach has yielded prolonged survival in poor prognosis patients. In both studies, the radionuclide was attached to an antibody that recognized a tumor-associated antigen.

In treating late-stage breast cancer patients (with measurable liver or bone metastases), long-lived alpha particle emitters are required to reach distant metastases that have developed their own vasculature. One of the most promising such radionuclides, Ac-225 has a 10-day half-life and results in intermediates that yield a total of 4 alpha particles. This radionuclide is shown to be 1000-fold more effective than Bi-213 (3), in animal studies, however, it has also proved to be far more toxic. The increased efficacy and toxicity are a result of the alpha-particle emitting intermediates. When these are confined to the target cells, efficacy is increased, when they distribute throughout the body, toxicity is increased. This is a fundamental difficulty if antibodies are to be used as the targeting vehicle since the bond holding the Ac-225 atom to the antibody will be broken after decay of Ac-225. This will leave the first daughter in the decay chain free to distribute throughout the body where it will decay and subsequently yield additional alpha emissions to normal organs from subsequent daughter decays (4, 5). In short, of the 4 alphas, only the first one, originating from decay of Ac-225 contributes to the tumor dose, the remainder will distribute throughout normal tissue to increase toxicity.

In this concept award we proposed to investigate the feasibility of using liposomal vesicles to encapsulate and retain Ac-225 and its radioactive daughters for delivery to tumor sites. Our results have shown that there is a fundamental limitation to this approach because of the 100 nm recoil distance of daughter nuclei following alpha-particle emission. This limitations requires the use of larger liposomes for adequate retention of daughters. The requirement for larger liposomes, in turn, limits application of this approach to locoregional administrations.

BODY

The studies described below were carried in collaboration with Prof. James L. Thomas of the Chemical Engineering Dept. at Columbia University, School of Engineering and Applied Science.

Materials and Methods

Actinium chloride was a generous gift from Drs. David A. Scheinberg and Michael R. McDevitt at Memorial Sloan-Kettering Cancer Center, New York, NY. The lipids L-α-phosphatidylcholine 95% (PC),

1,2-dipalmitoyl–SN-glycero-3-phosphocholine (DPPC), and rhodamine-labelled phosphatidylethanolamine (r-PE) were purchased from Avanti Polar Lipids (Alabaster, AL). Cholesterol, NTA, diethylene-triaminepentaacetic acid (DTPA), sephadex G-50 and sodium chloride were purchased from Sigma (St. Louis, MO). Indium-111 radionuclide was from PerkinElmer (Boston, MA). In all experiments, double deionized water was used (Millipore filtration system).

Vesicle Preparation

Aliquots of PC, cholesterol (2:1 mole ratio), and r-PE (1:350 wt:PC) in chloroform solutions were dried to a thin film under a stream of nitrogen gas, and then were put in vacuum overnight to remove all traces of solvent. (The tracer r-PE was included to facilitate tracking of the lipids through the various chromatography columns.) For chemical loading experiments, lipids were resuspended in 1mM DTPA (or NTA) in 5 mM HEPES or sodium phosphate buffered isotonic saline (0.9 wt% NaCl, pH 7.4). (The selection of buffer did not affect loading efficiency.) For passive loading, the lipids were resuspended in a buffer containing actinium-DTPA complexes (1 mM DTPA). The suspension was extruded 19 times through two polycarbonate filters with defined pore sizes, using the Liposofast extruder (AVESTIN, Canada). Unentrapped DTPA was removed by size exclusion chromatography (SEC) in a Sephadex G-50 packed 1.0x10 cm in sodium phosphate isotonic saline (for indium) or HEPES isotonic saline (for actinium).

Chemical Loading

The loading protocol for indium is published elsewhere.(6, 7) Briefly, to 1 mL of vesicle suspension, 100 μL of $InCl_3$ in 3 mM HCl and 100 μL of oxine in 1.8 wt % NaCl / 20mM sodium acetate (3 μL of 11 mM oxine in EtOH stock solution added to the acetate buffer.) After loading, unentrapped ^{111}In-oxine complexes were removed by AG1X-8 phosphate ion exchange column. The same protocol was used for actinium, except that the ionophore A23187 was included in the vesicle preparation.

Activity

Activity was measured using a Packard Instrument B5003 gamma counter (Downers Grove, IL.) At steady state, the decay rate of each nuclide will be the same, so that the decay rates and concentrations can be measured by measuring any member of the chain. Only francium and bismuth emit gamma rays, however.

Indium, an actinium "analogue"

Radioactive indium (^{111}In) has been loaded into lipid vesicles by using a combination of a membrane transporter (ionophore) and an entrapped indium chelator (6, 7) . Good results have been reported in the literature using the ionophore oxine and the chelator DTPA. We have successfully reproduced these results, as shown in Figures 1 and 2. To determine the stability of indium entrapment, indium-loaded 100 nm vesicles were run over a size-exclusion column (Sephadex G-50 1x10 cm) at various times after loading. The size exclusion column effects a clean separation between the large vesicles and free indium, as shown in Figure 1. The cumulative ^{111}In recovered in the vesicle fractions is shown by the dashed lines, and is approximately 80% for newly-prepared vesicles (3 days after loading) and vesicles stored for 17 days at 4°C. (The 20% loss, independent of vesicle age, is likely caused by slight vesicle disruption during the column chromatography.)

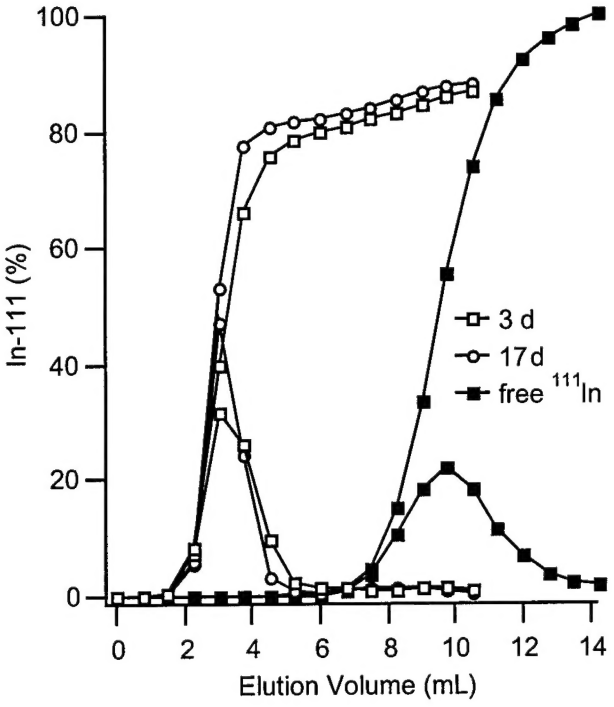

Figure 1. Elution profiles of indium-loaded vesicles 3 days and 17 days after preparation. The vesicles elute from the size-exclusion column much more rapidly than free indium, run as a separate control. The cumulative indium recovery from the vesicle fractions is shown as dashed lines, and is ca. 80%, regardless of vesicle storage time.

A summary of more than two dozen measurements is shown in Figure 2. Vesicles retained ^{111}In well for at least a month, regardless of whether storage was at 4°C or at 37°C: in fact, there is no evidence for *any* increased leakage with time.

DTPA is a strong indium chelator ($K_D=10^{34}$ M^{-1},(6)). Even if indium were able to permeate the membrane, most indium might remain within the vesicle if the DTPA is stably entrapped. To test this possibility, the entrapment stability experiments were also conducted with 1 mM DTPA in the exterior buffer, thus removing the chemical DTPA gradient that might facilitate indium retention. The results of these measurements were indistiguishable from those made without external DTPA (triangles and dashed line, Figure 2.) We conclude that ^{111}In and ^{111}In-DTPA complexes are stably entrapped within these vesicles, and cannot permeate the membrane on a one-month timescale.

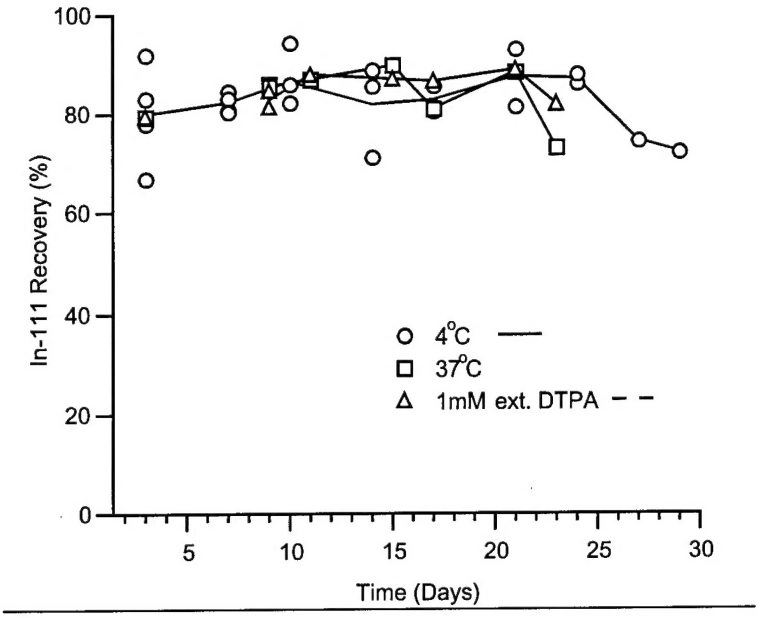

Figure 2. Indium retention in the vesicle fractions as a function of vesicle storage time, for different storage conditions. The solid, dotted, and dashed lines show the mean value for measurements made on duplicate samples.

Actinium

Actinium-225 decays with the emission of an alpha particle; alpha emissions are difficult to detect and require scintillation fluids. Fortunately, the decay chain of actinium includes francium and bismuth, both of which decay by gamma emission, with branching ratios of 10% and 16% respectively. Under steady state conditions, the decay rate of each species in the decay chain must be equal; thus, at steady state, the decay of either francium or bismuth can be used to determine the actinium concentration. A typical experiment is shown in Figure 3. Vesicles were prepared as described in Materials and Methods, by extrusion through 800 nm pore filters. The hydration buffer contained DTPA-chelated ^{227}Ac, some of which becomes physically entrapped in the vesicles during their formation ("passive loading.") Unentrapped actinium was then removed using size-exclusion chromatography. In the experiment shown, the vesicles were rechromatographed after 74 hours, and the ^{221}Fr and ^{213}Bi gamma emissions from the vesicle fraction were measured. The low initial ^{213}Bi activity shows that bismuth has been largely lost from the vesicles: had bismuth been retained, the radioactivity of the vesicle fraction would have been at steady state. In this newly-separated

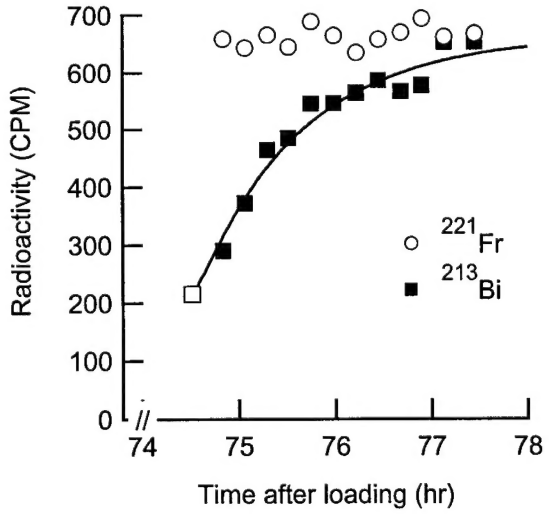

Figure 3. After vesicle separation, gamma-ray counts from francium or bismuth approach steady-state values, which are then indicative of the amount of entrapped actinium. The time at which vesicle separation began is shown with the square symbol.

vesicle fraction, the bismuth activity rises as ongoing actinium decay gradually brings the entire fraction into steady state. (The kinetics of the rise in bismuth activity are nearly monoexponential with a $t_{1/2}$ equal to the bismuth half-life; decay kinetics below.) The steady state radioactivity of bismuth, when corrected for the instrumental response (48% detection efficiency) and branching ratio (16%) are equal to the steady state activity of actinium in the fraction. In the example shown, this is ca. 8500 disintegrations per minute = 141 Bq.

Francium gamma emission was also measured. Unfortunately, the kinetics of francium equilibration is so rapid ($t_{1/2}$ is ca. 5 min) that no reliable estimate of francium entrapment could be made. The measured steady state francium decay rate was found to be about the same as that for bismuth – a fortuitous consequence of having the same product of detection efficiency (80%) and branching ratio (10%).

Measurements of steady state ^{213}Bi activity in vesicle fractions separated from the parent vesicle population at different times allow for an estimate of the stability of actinium entrapment, Figure 4. Here, steady state activity has been plotted vs. time of (second) separation, for 400 nm vesicles. Because actinium has a ten-day half life, the activity in the vesicle fraction will diminish with time, even if no leakage occurs. This decay in activity is plotted as the solid line, with the intercept given by the original activity (after first vesicle separation). At various times after vesicle preparation, the vesicles were rechromatographed, and the steady state ^{213}Bi activity was measured (shown as circle symbols). This activity decays with the expected actinium half life. The ^{213}Bi activity is also shown as a fraction of the expected activity in the absence of vesicle leakage, square symbols. Independent of time, the activity recovered in the vesicle fraction is about 90% of ideal. The 10% loss may be caused by the chromatographic separation itself. *Importantly, actinium is retained within these vesicles for more than one month.*

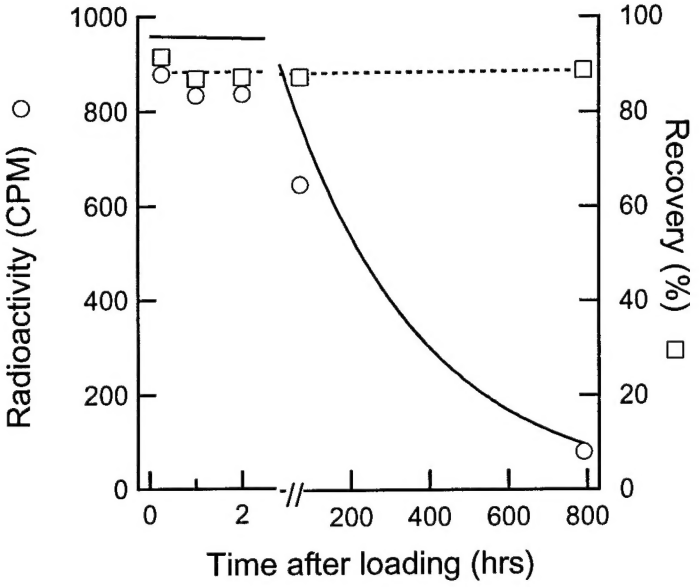

Figure 4. Steady state ^{213}Bi activity from vesicle fractions separated at various times after loading. The recovered activity is essentially constant at nearly 90% of that originally loaded, when normalized to the actinium decay.

The loss of ^{213}Bi from vesicles was then studied, as a function of vesicle preparation protocol. Bismuth could leave the vesicle interior either through passive permeation, or through nuclear recoil occurring during the decays of actinium, francium, or astatine. These species are likely to be ionic (Ac^{+3}, Fr^{+1}, At^{-1}, Bi^{+3}) and are thus expected to

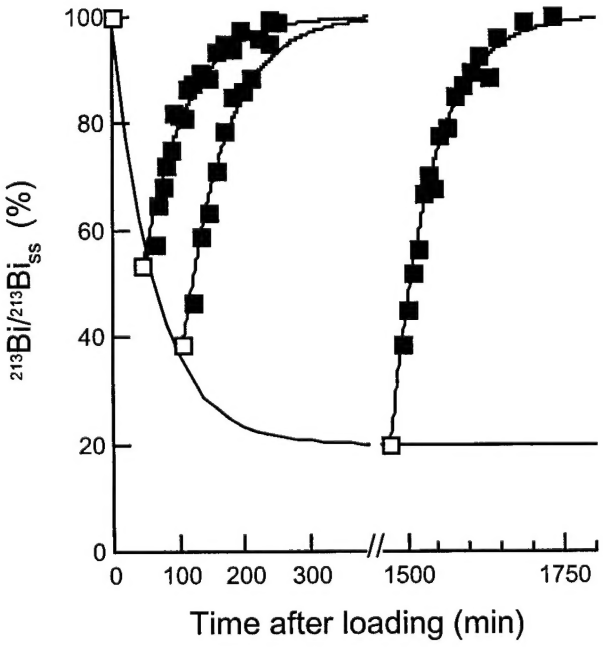

Figure 5. At several times after preparation (□), vesicles were rechromatographed and the ^{213}Bi activity was measured in the pooled vesicle fractions (■). The decay curve and the recovery curves can be fit with a single parameter, the fraction f of disintegrations that result in daughter ejection. These vesicles were prepared by extrusion through 400 nm pore filters. $f \approx 0.2^{1/3}$.

permeate lipid membranes very slowly (8). Regardless of the mechanism of loss, larger vesicles should show better ^{213}Bi retention than smaller vesicles. In these preliminary studies, we have prepared vesicles by extrusion through nuclear track-etch membranes with differing pore sizes. Larger pores are known to produce vesicle populations with larger mean diameters (9). (The vesicle diameter may be larger or smaller than the pore diameter, possibly depending on variables such as lipid composition, and extrusion pressure or rate. No systematic study has been reported in the literature.) Actinium was passively entrapped in the vesicles during extrusion, and then unentrapped actinium was separated chromatographically. For each vesicle population, measurements of ^{213}Bi activity were made following rechromatography at various time points, Figure 5. The recovery of ^{213}Bi activity to steady state levels has been discussed above; the decrease in initial ^{213}Bi activity reflects the rapid loss of bismuth from vesicles. Note that the vesicles initially entrap steady-state concentrations of all species in the decay chain, since loading is passive. The ^{213}Bi decay curve and the recovery curve can both be well fit by assuming that a fixed fraction f of nuclear decays results in daughter ejection. When vesicles are chromatographed at long times, the fraction of bismuth remaining within them approaches f^3.

Because the chromatographic separation requires time (ca. 10 minutes) and because of instrumental issues, it is not possible to determine the ^{213}Bi activity "at the instant of separation." We have thus extrapolated the ^{213}Bi activity measurements backwards ca. 30 minutes, to the time at which separation was initiated, using the fit recovery curve. These extrapolated points are shown as square symbols in Figures 3 and 5. The exact ^{213}Bi activity is likely to lie between this extrapolated point and the first experimental observation. In Figure 6, the steady-state bismuth retention is shown as a function of the pore size of the filters used in vesicle preparation. The top point of each bar is the first experimental measurement, while the bottom is the extrapolation to the beginning of separation. The retention is higher for the larger vesicles, as anticipated. As mentioned above, both the kinetics of ^{213}Bi activity changes and literature values for ionic permeability support the contention that this loss of bismuth is caused by nuclear recoil, rather than passive permeation. Note that the loss is not likely to be caused by membrane damage from the ionizing radiations: actinium remained stably entrapped for more than one month.

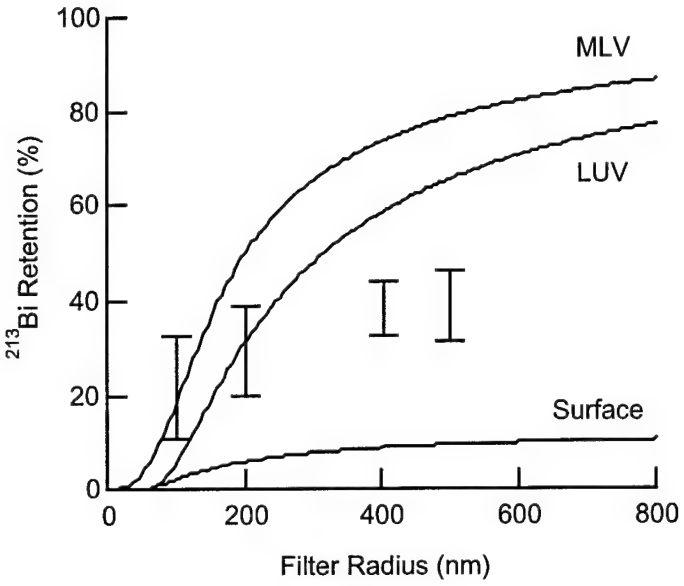

Figure 6. Vesicles prepared by extrusion through filters with larger pores show improved bismuth retention. Retention is not as high as theoretically achievable. Vesicles may be somewhat smaller than the filter pore size; alternatively, transient binding of radionuclides to the vesicle surface could easily reduce retention.

Although we have not sized the vesicles used these experiments, it is useful to compare these experimental results with expectations from nuclear theory and geometry. The nuclear recoil distances of francium, astatine, and bismuth alpha decays are not well established in aqueous media, but estimates to within 10-20% can be made (10). Using a standard computer model (SRIM, Stopping and Range of Ions in Matter, James Ziegler, http://www.srim.org/), the range of these recoils can be estimated as 81.7, 86.5, 94.7 nm. Since these differ by less than the experimental uncertainties (both in the recoil range estimates and in our experimental measurements), we have used a single recoil distance to simplify further calculations. Three models have been considered. The first is the "LUV", or large unilamellar vesicle model, in which it is assumed that each entrapped radionuclide is distributed uniformly within the aqueous volume of a vesicle. Thus, each disintegration has a fixed probability f for daughter ejection. The probability of ejection for a disintegration that occurs a distance r from the center of a vesicle of radius r_v may be calculated from geometry, as shown in Figure 7a. The probability f is calculated by averaging the ejection probability over the possible locations of the radionuclide, r :

$$f_{r,r_d} = \begin{cases} 1 - \dfrac{2rr_d - r_v^2 + r^2 + r_d^2}{4rr_d}, r > r_v - r_d \\ 1, r < r_v - r_d \end{cases}$$

$$f = \int_0^{r_v} 4\pi f_{r,r_d} r^2 \, dr \Big/ \tfrac{4}{3}\pi r_v^3$$

where r_d is the recoil distance. Retention of bismuth requires three successive decays without escape, so that the probability is f^3. This is shown in Figure 6 as the theoretical line "LUV", calculated assuming a recoil distance of 87.6 nm (average recoil distance in water calculated using SRIM.)

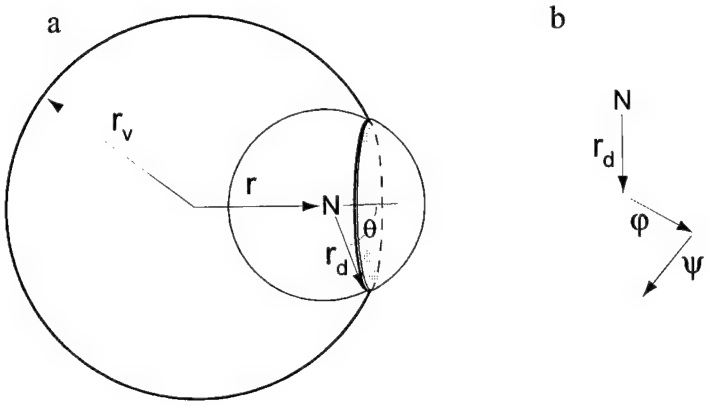

Figure 7a. Geometry for daughter retention from the decay of a radionuclide, N, a distance r from the center of a vesicle of radius r_v. The daughter will recoil to a random position on the surface of the small sphere, defined by the recoil radius r_d. The fraction of this sphere outside the vesicle is the probability of daughter escape. b: Without diffusion between successive decays, ^{213}Bi will recoil a total distance from the actinium parent given by the vector sum of the successive decays.

Larger vesicles are likely to be multilamellar (9). This may prevent the free diffusion of daughter nuclides within the vesicle. In the absence of diffusion, the loss of bismuth is better modeled by three recoil events, as shown in Figure 7b. In this case, the total recoil distance r_t is determined by the two azimuthal angles φ, ψ, which will assume random values (uniformly distributed in $\alpha = \cos\varphi$, $\beta = \cos\psi$) and the law of cosines:

$$r_t = r_d \cdot \left\{3 + 2\alpha + 2\beta\sqrt{2+2\alpha}\right\}$$

The calculation then follows the single decay LUV calculation above, with additional integrations over the azimuthal angles:

$$p = \int_{-1}^{1}\int_{-1}^{1}\int_{0}^{r_v} 4\pi f_{r,r_t} r^2 dr\, d\alpha\, d\beta \Big/ 4 \cdot \tfrac{4}{3}\pi r_v^3$$

This calculation shows that a slightly higher retention probability should be achievable in multilamellar systems. The result is shown in the Figure as the line marked MLV.

The experimental result is significantly below the theoretically achievable retention, for either model. A likely explanation is simply that the size of the vesicles is significantly smaller than the filter pore sizes, in our preparations. We plan to test this hypothesis with electron microscopy and quasielastic light scattering estimates of vesicle sizes. In addition, the assumption that radionuclides distribute uniformly throughout the vesicle may be unwarranted. It is possible that they transiently but significantly associate with the vesicle surface. The limiting bismuth retention, if nuclides are exclusively at the vesicle surface,is calculated from the "LUV" retention model by including $\delta(r\text{-}r_v)$ within the integral over r and normalizing appropriately. The result for three successive

"surface" disintegrations is also shown in the Figure: the maximum bismuth retention approaches $1/2^3$ = 12.5% for large vesicles, as expected. (Half the recoils from the surface of an infinitely large vesicle would result in daughter ejection.) Clearly, if even a fraction of the daughters of actinium (or of actinium itself) associate with the vesicle membrane, the bismuth retention will be significantly reduced.

Chemically-Driven Entrapment of Actinium

Although passive loading is adequate to carry out experiments on actinium daughter retention, application of vesicle-entrapped actinium *in vivo* will probably require higher loading efficiencies than we have obtained with this method (typically 5-10%.) In preliminary experiments, we have *not* been able to achieve high loading levels of actinium using the ionophore oxine. Vesicles loaded using DTPA and oxine (6 d incubation) show an initial loading efficiency of about 18%, but most of the actinium is lost within three days after loading, Figure 8. Doubling the oxine concentration did not improve loading. We hypothesize that the phosphates of the lipid headgroups may participate with oxine in forming an actinium-binding complex; evidence of such complexes may be found in the work of Hwang (6). If these complexes do not translocate (or 'flip-flop') across the membrane, the ionophore might function only to bind actinium,

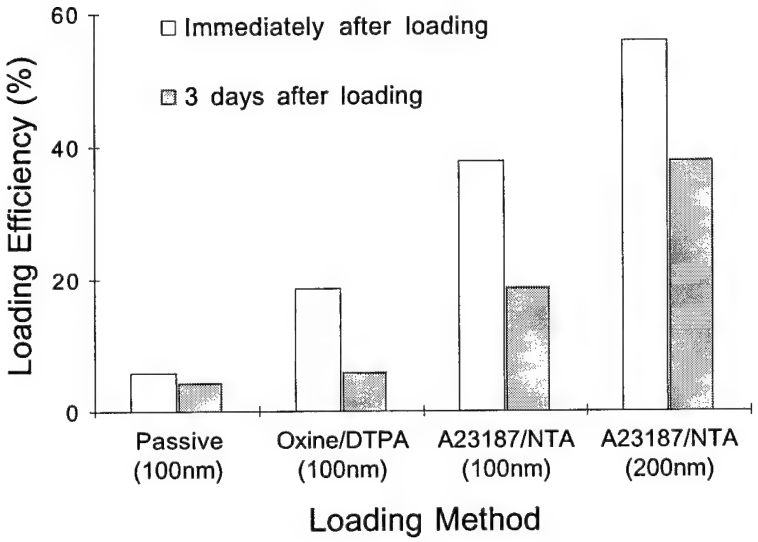

Figure 8. Loading efficiency (% of total actinium recovered in the vesicle fraction) for different loading protocols. Over the first three days, about 13-19% of the actinium is released from chemically loaded vesicles; the remainder of the actinium remains within the vesicles for an extended

not to deliver it to the internal DTPA.

An alternative ionophore, A23187, has been used with indium and the chelator NTA for vesicle loading (11). We studied whether the A23187/NTA combination would provide for better chemical loading of actinium, and this was indeed the case, as shown in the Figure. In addition, varying the concentration of A23187, from 0.04 mol % (to PC) up to 0.4 mol %, did *not* give better loading.

Encouragingly, chemical loading protocols appear to work as well for larger, probably multilamellar vesicles as for those that are smaller and unilamellar, Figure 9. Loading was also equally effective with saturated dipalmitoyl phosphatidylcholine containing

vesicles. (Results not shown.) Overall efficiencies of chemical loading protocols may be improved by varying loading parameters such as temperature, time, and concentrations of ionophores.

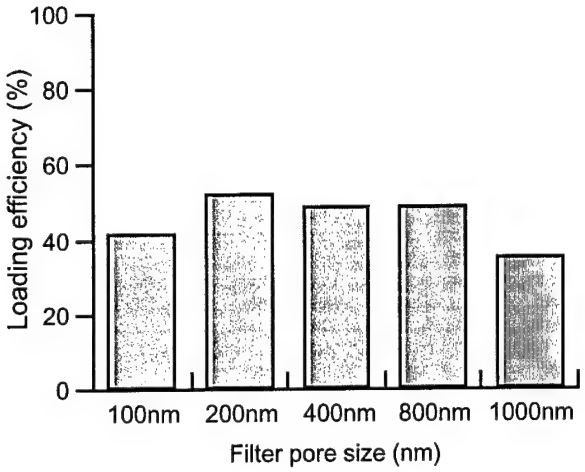

Figure 9. Loading efficiency using A23187/NTA is largely independent of vesicle size.

Kinetics of Serial Disintegrations

The activity curves in Figures 3 and 5 appear monoexponential, but they are not. An exact solution for the ^{213}Bi activity can be obtained by solving the coupled differential equations that describe the actinium decay chain. We construct a (mathematical) vector of the concentrations of each species:

$$\vec{C} = \begin{bmatrix} C_{Fr} \\ C_{At} \\ C_{Bi} \end{bmatrix}$$

We omit actinium, which may be taken as essentially constant over the five-hour course of a bismuth measurement. The differential equation describing the time evolution is then:

$$\frac{\partial \vec{C}}{\partial t} = \vec{\vec{K}} \cdot \vec{C} + k_{Ac} C_{Ac} \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix}$$

where:

$$\vec{\vec{K}} = \begin{bmatrix} -k_{Fr} & 0 & 0 \\ +k_{Fr} & -k_{At} & 0 \\ 0 & +k_{At} & -k_{Bi} \end{bmatrix}$$

The second term in the equation represents the formation of francium from the decay of actinium. We have also assumed that the branching ratios are 100%; the actual branching ratios are all >97.9% (12).

This equation may be solved using Laplace transform methods (MATLAB software) for given initial conditions. Assuming a fixed probability for daughter retention (i.e. the "LUV" model described above), the initial condition after vesicle separation is:

$$\vec{C}_0 = \begin{bmatrix} f \cdot C_{Fr,SS} \\ f^2 \cdot C_{At,SS} \\ f^3 \cdot C_{Bi,SS} \end{bmatrix}$$

Where $C_{X,SS}$ is the steady-state concentration of species X. For example, the steady-state concentration of francium is given by:

$$\frac{\partial C_{Fr}}{\partial t} = 0 = +k_{Ac}C_{Ac} - k_{Fr}C_{Fr}$$

The steady-state concentrations of the other species may be similarly obtained. With the initial condition established, the solution to the time evolution after vesicle separation depends only on f and $C_{Ac.}$; the latter is simply a scale factor that $\rightarrow 1$ on normalization.

Because the half-lives of francium and astatine are so short compared with bismuth, the bismuth kinetics are dominated by the bismuth half-life. Only at the very earliest part of the fit curve can any deviation from single exponential behavior be seen; our experimental set-up does not permit a direct test of this deviation.

The loss of bismuth from within a vesicle can be similarly modeled (LUV model), by modifying the matrix K:

$$\vec{K} = \begin{bmatrix} -k_{Fr} & 0 & 0 \\ +f \cdot k_{Fr} & -k_{At} & 0 \\ 0 & +f \cdot k_{At} & -k_{Bi} \end{bmatrix}$$

and replacing k_{Ac} by $f\,k_{Ac}$ in the kinetic equation for francium. After physical entrapment, we assume all species are present at steady-state concentrations.

This exercise has shown that bismuth dynamics can be well-modeled by a single exponential decay or recovery, in spite of the underlying complexity of the coupled decay kinetics.

KEY RESEARCH ACCOMPLISHMENTS

- Demonstrated prolonged and stable retention of In-111 in liposomal vesicles under various challenge conditions (external DTPA, 37 °C, 4 °C).
- Developed a methodology for high efficiency encapsulation of Ac-225 in liposomal vesicles
- Demonstrated prolonged (> 1 month) and stable retention of Ac-225 in liposomal vesicles.
- Demonstrated that the loss of Bi-213 is dependent upon vesicular diameter and that loss is likely due to ejection by recoil following alpha-emission of the parent.
- Developed a mathematical model that may be used to describe loss of daughters due to recoil and that predicts high theoretical retention of Bi-213.

REPORTABLE OUTCOMES

Thomas JL, Lin H, Palm S, Sofou S, Sgouros G. Entrapment of the Alpha-emitter Actinium and its Daughters within Liposomes. Abstract submitted to The Biophysical Society Annual Meeting, February 2002, San Francisco, CA.

CONCLUSIONS

Work carried out to date has demonstrated the feasibility of stably entrapping Ac-225 in liposomal vesicles. Daughter retention, however, is limited by the recoil distance of daughter nuclei. This fundamental limitation requires vesicle diameters that are substantially greater than 100 nm. Liposomal vesicles that are greater than 100 nm in diameter are not optimal for intravenous injection because of inadequate tumor accumulation, high reticuloendothelial system accumulation and short half-life in circulation. Our focus, has therefore, changed to examining feasibility for use in loco-regional administrations. In the remaining year of the proposal we plan on optimizing retention so that it approaches the theoretical limit of 75% for 400 nm diameter vesicles. We will examine efficacy and possible improvements in toxicity using an animal model of intraperitoneal disease that can also be targeted by Ac-225-labeled Herceptin.

SO WHAT? These preliminary studies have shown that daughter retention depends upon liposomal vesicle diameter and that > 20% retention requires vesicles greater than 100 nm in diameter. Our focus, therefore, is now on locoregional application, including, for example, intrahepatic and intraperitoneal administrations.

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APPENDIX

Thomas JL, Lin H, Palm S, Sofou S, Sgouros G. Entrapment of the Alpha-emitter Actinium and its Daughters within Liposomes. Abstract submitted to The Biophysical Society Annual Meeting, February 2002, San Francisco, CA.

Actinium-225 is the parent in a chain of four alpha emitters, and is thus a good candidate radionuclide for cancer therapy, provided the radioactive daughters can be localized to the tumor site. Towards this end, we have successfully entrapped actinium within phosphatidylcholine-cholesterol liposomes formed by the extrusion method. The radioactivity of two daughter products, francium and bismuth, are easily measured in a gamma counter, because these nuclides also decay by gamma emissions. Thus, we are able to use the radioactivity of these daughters to follow the entrapment both of actinium and of the individual decay products. Our results show that the dominant mechanism for loss of the daughters is the kinematic recoil that occurs on alpha emission. As a consequence, larger liposomes are able to retain a larger fraction of the daughters, especially bismuth. The loss of the daughters was modeled for large unilamellar and multilamellar vesicles, and the predictions of each model are compared to the experimental observations. Prospects for therapeutic use of liposomes and liposomal constructs will be discussed.